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Synopsis of Research Topic

"Inulin-Based Polymeric-Metallic Nanoparticles for Photothermal Therapy Coupled with Curcumin for Cancer Theranostics Applications"

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Abstract of Proposed Work Plan/ Problem

Topic of Research - **Inulin-Based Polymeric-Metallic Nanoparticles for Photothermal Therapy Coupled with Curcumin for Cancer Theranostics Applications**

❖ Introduction:

Cancer is a specific supreme cause of death worldwide. According to recent reports, it is the second major death conviction worldwide. According to a report by the American Cancer Society, by 2030, 21.7 million new cancer cases will be diagnosed. Current cancer treatment strategies include chemotherapy, radiotherapy, surgery, immunotherapy, stem cell transplantation, etc. But these conventional strategies have significant adverse side effects, and there is a vast scope for developing new treatments. In this context, nanotechnology can play a vital role in drug delivery and imaging, targeting specifically cancerous cells without affecting healthy normal cells. The tumour microenvironment shows enhanced permeability and retention effect (EPR effect), where the nanoparticle system preferentially accumulates at the tumour site. Due to poor tumour vasculature, the nanoparticle system stays in the tumour tissue for extended periods due to poor lymphatic drainage [1]. This makes nanoparticle systems an ideal candidate for cancer theranostics!

Photothermal therapy (PTT) is a novel art of war which uses near-infrared (NIR) photo-absorbers to create heat for the thermal attrition of carcinoma cells upon irradiation by NIR lasers [2]. PTT has many advantages over conventional therapies, which include minimal invasiveness and precise spatiotemporal specificity. PTT's efficacy depends upon transforming light to adequate heat using photo-absorbers, mostly nanoscale photo-absorbers. Novel biodegradable, versatile nanoparticle systems are being explored for photothermal efficacy, *in vivo* degradation and clearance. NIR dyes for PTT, like IR-780, have limited clinical application due to their hydrophobicity and dose-dependent toxicity [2]. However, encapsulation of this dye within nanosystems increases its aqua dispersibility and decreases its toxicity; such factors, in combination with anti-cancer drugs or anti-cancer plant extracts, will maximise sanative efficacy resulting in a synergistic approach to cancer treatment.

Curcumin-rich extracts are known for their anti-cancer activity due to their anti-oxidant behaviour, mutagen trapping, induction of apoptosis & regulation of detoxification pathways [3]. Also, natural curcumin shows anti-genotoxic & anti-mutagenic activity and bacterial & mammalian cell-based assays [4]. However, the hydrophobicity of curcumin extracts restricts the likelihood of enhanced therapeutic efficacy due to low bioavailability and reduced cellular uptake. Curcumin-rich extract exhibits anti-proliferative and pro-apoptotic properties. Nanotechnology plays a vital role in boosting the dispersibility and bioavailability of hydrophobic drugs.

Inulin is a naturally occurring polysaccharide from plants like garlic, chicory, onion etc. Inulin is also FDA-approved, non-toxic and has demonstrated animal cancer prevention [3]. Conjugation of inulin with anti-cancer drugs has shown increased drug stability towards light, temperature, chemical agents and hydrolysis. Hence, inulin can be a carrier for medicines or plant extracts. Also, the inulin plant extract nanosystem can be used for photothermal therapy as the release of the extract can resist the recurrence of a tumour at the treated site. Also, some researchers have shown the role of inulin in inhibiting colon-rectal pre-neoplastic wounds indicating that inulin may defeat colon oncogenesis.

The only problem in using inulin as a carrier is its recognition by the reticuloendothelial system and quick renal clearance, decreasing its stay in the bloodstream [1]. Hence, conjugates of inulin using different polymers can be prepared to prevent its recognition by the reticuloendothelial system (RES) & lengthen bloodstream circulation, making it a suitable carrier for anti-cancer drugs or plant extracts.

In this study, we will use a biocompatible INULIN-PEG in combination with a metallic nanosystem to encapsulate curcumin extract [10]. The fluorescence of curcumin can be used to understand the cellular uptake and drug release profiles. This nanosystem will be amalgamated with NIR dye IR-780 to make it photo-thermally active. Combining photothermal therapy with a triggered release of curcumin can be used as a coadjuvant approach for cancer therapy.

❖ Purpose of Proposed Research work:

There is a huge demand for cancer therapies that can effectively eliminate solid tumours and residual metastatic nodules, preventing the recurrence of a tumour. Conventional treatments like chemotherapy, radiotherapy, surgery, stem cell transplantation, hormonal therapy, etc., have a drawback of tumour recurrence. To overcome this limitation, using photothermal treatment in combination with adjuvant therapy can be an effective approach as the chances of recurrence of a tumour can be minimized [10]. PTT is very effective in curing local tumours. By target-oriented administration of photo-absorbers and minimally invasive near-infrared (NIR) radiation, thermal ablation using PTT can be controlled to minimize damage to non-cancerous tissues.

PTT can be combined with adjuvant therapy to reduce the chance of tumour recurrence. The curcumin-rich extract can be used as an adjuvant as it was reported to have anti-cancer activity, and curcumin-rich derivatives are known to have imaging advantages [3], [4]. The fluorescence of curcumin can be attuned to monitor cellular uptake and drug release. To deal with the hydrophobicity of chlorophyll extract, the extract is encapsulated within a polymeric nanosystem, i.e. inulin nanosystem.

Inulin is an FDA-approved biopolymer for intravenous injection. It represents a suitable scaffold for biocompatibility and coadjuvant anti-cancer effect to design knick-knack anti-cancer carrier systems [5]. Inulin still needs to be used for photothermal therapy on a large scale. Inulin can be modified using PEG to prevent recognition by the RES & for prolonged bloodstream circulation. In contrast, curcumin can increase cellular uptake more in cancer cells than in normal cells. The nanosystem, i.e. INULIN-PEG-CURCUMIN, in combination with the metallic portion, has the added advantage of accumulating specifically at the tumour site due to the perforated vasculature of the affected tissues and imperfectly developed lymphatics (EPR) effect [1].

Thus, a novel nanosystem (INULIN-PEG) encapsulating curcumin-rich extract conjugated with NIR dye IR-780 can be used for synergistic therapy, including PTT and adjuvant curcumin in the treatment of cancer as well as prevention of its recurrence.

❖ Literature Review & Development in the Cancer Research:

The following literature review is important for the proposed research work.

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- **JinFeng Liao *et al.***

Studied combined cancer photothermal chemotherapy and reported using gold nanostructures and Doxorubicin (DOX) for combined PTT/chemotherapy. Therein gold nanostructures were used as photo-absorbers for PTT, and DOX was used as an anti-cancer drug for chemotherapy, enabling their synergistic combinational therapy [6]. For targeted therapy, the choice of carrier is most crucial. The carrier molecule should have properties like biocompatibility, targeting cancer cells without affecting normal cells, and enhanced cellular uptake.

- **Licciardi *et al*.**

  Worked on amphiphilic inulin graft copolymers for doxorubicin delivery. They reported that inulin could be conjugated with hydrophilic chains like PEG and hydrophobic groups like curcumin for enhanced drug delivery [5]. They used PEG as a hydrophilic outer shell, as it provides adequate steric protection and prevents recognition by the reticuloendothelial system (RES), resulting in prolonged bloodstream circulation. In contrast, curcumin was used to provide hydrophobic interaction and enhance the synergistic effect by improving the anti-cancer activity of the delivered drug.

- **A Kunwar *et al*.**

  Worked on encapsulating curcumin-rich biomolecular fractions into polymeric nanosystems combined with PTT [7]. They reported the successful incorporation of hydrophobic curcumin into PLGA nanoparticles. They made it photo-thermally active using IR-780 and evaluated the PTT-mediated release of the biomolecular fraction. They showed that incorporating Curcumin/IR-780 into PLGA NPs coupled with PTT showed synergistic cytotoxicity compared to PTT or curcumin treatment alone.

- **Sawa T *et al*.**

  Studied tumour-targeted chemotherapy using xanthine oxidase-polymer conjugate and reported that conjugation of XO with PEG significantly enhanced tumour-targeting efficacy and anti-tumour activity [8]. They said that using PEG enhances the EPR effect; hence, the tumour-targeting efficacy of the conjugate is increased [9].

The Development in cancer research includes combinational (Bimodal) therapy using PTT and adjuvant chemotherapy is an excellent approach to cancer treatment and prevention of its recurrence. It shows that curcumin can be used as an adjuvant with PTT for synergistic therapy. Inulin is an effective carrier for targeted therapy due to its biocompatibility. It can be conjugated with PEG and Curcumin to increase cellular uptake due to its amphiphilic nature, increased EPR effect and prevention of recognition by RES. Combining different therapeutic techniques for cancer treatment has shown great potential in clinical and fundamental studies. Photothermal therapy can be combined with conventional therapies to exert synergistic therapeutic efficacy for treating cancer metastasis.

❖ Research Gap (s):

Even with many recent advances in cancer research, there are still critical gaps in our fundamental understanding of the cause, pathogenesis, and natural history of many different types of cancers, especially mediators of the transition from premalignant to preinvasive to invasive phenotypes. The main obstacle hindering photothermal therapy from becoming a common and reliable treatment for cancer in the healthcare industry is the inefficient and inconsistent ability of materials used in the involved procedures. This kind of research gap generates the scope for researchers.

❖ Importance of the Study:

International Status: Cancer is a chief cause of demise globally, accounting for nearly 10 million deaths in 2020, or almost one in six reasons for the loss of life. The most ordinary cancers are breast, lung, colon, rectum & prostate cancers. Around one-third of deaths from cancer are due to tobacco consumption,

high body mass index, alcohol drinking habit, low fruit and vegetable ingestion, and lack of physical workout. Cancer-causing infections, such as human papillomavirus (HPV) and hepatitis, are accountable for roughly 30 % of cancer cases in low- and lower-middle-income countries. Numerous cancers can be cured if detected early and treated fruitfully [12].

National Status: The approximate integer of occurrence of cancer cases in India for 2022 was developed to be 14,61,427 (crude rate: 100.4 per 100,000). In India, one out of nine people will probably develop cancer in their lifespan. Breast and lung cancers were the prime cancer sites in males and females. Among the babyhood (0-14 yr) cancers, the primary site was lymphoid leukaemia (boys: 29.2 % & girls: 24.2 %). The prevalence of cancer instances is estimated to increase by 12.8 per cent in 2025 compared to 2020 [11].

Regional Status: The states like Mizoram, Manipur, and Arunachal Pradesh are states with the highest cancer rates in India. 14.6 lakh new cases in 2022. 14.2 lakh in 2021, and 13.9 lakh in 2020. Deaths due to cancer increased to about 8.08 lakh in 2022 from 7.9 lakh in 2021 and 7.7 lakh in 2020. One in nine Indians will develop cancer during their lifetime. Overall, Uttar Pradesh recorded the highest number of 2.01 lakh cancer cases in 2020, followed by Maharashtra with 1.16 lakh cases and West Bengal with 1.08 lakh cases.

Scope of Study: It seems reasonable to project even more accelerated improvements in the treatment of cancer over the coming decade. In 10 years, it may be possible to detect cancer before visible tumours have formed and treat it with early intervention and prevent further development. In the future, advances in sensors, contrast agents, molecular methods, and artificial intelligence will help detect cancer-specific signals in real-time. To reduce the burden of cancer on society, risk-based detection and prevention need to be cost-effective and widely accessible.

❖ Objectives of the present work:

- Preparation of Curcumin/IR-780 loaded INULIN-PEG polymeric-metallic nanosystem.
- Characterization of INULIN-based polymeric metallic nanoparticles.
- Evaluation of cellular uptake and *in vitro* imaging of INULIN-Curcumin nanoparticles using cancer cell lines.
- Investigation of photostability of INULIN-Curcumin nanoparticles.
- Study the photothermal transduction efficacy and triggered release of Curcumin from INULIN-Curcumin nanoparticles.
- Study of Biocompatibility and PTT-mediated cytotoxicity of INULIN-Curcumin nanoparticles.
- To study the drug accumulation/efficacy profile under *in vivo* conditions.

❖ Hypothesis:

Conventional therapies for cancer treatment have some limitations like adverse effects on normal cells, recurrence of tumour post-therapy, etc. So, combinational treatment can be used to solve this problem. A biocompatible polymer like inulin can encapsulate plant extracts showing anti-cancer activity. Modifications can be made to the structure of inulin to increase its cellular uptake, prolong bloodstream circulation and enhance its anti-cancer properties using PEG and Curcumin. Also, conjugation of this system with NIR dye IR-780 can make it photo-thermally active for PTT-triggered drug release of the biomolecular extract. We hypothesise that incorporating Curcumin into the INULIN-PEG nanosystem coupled with PTT can be a synergistic approach for cancer treatment.

❖ Research Methodology:

The present research is completely focused on cancer theranostics applications with the help of nanoparticle treatment. The proposed Inulin-PEG polymeric metallic nanosystem will be biocompatible and without toxicity. This nanosystem will be entrapped with curcumin. INULIN-PEG-CURCUMIN polymeric-metallic nanosystem will be prepared using a multistep reaction; In the first step, Inulin will be combined with mono-methoxy PEG to form an INU-PEG copolymer. Curcumin-rich fractions will be made into nanoparticles using the INULIN-PEG copolymer by emulsion diffusion and solvent evaporation. Later, it will be combined with a suitable metallic entity for effective PTT treatment.

Four types of the combination will be made, only INULIN-PEG combination with metallic NPs, INULIN-PEG polymeric-metallic NPs loaded with CURCUMIN, INULIN-PEG-CURCUMIN NPs loaded with IR-780 and INULIN-PEG-CURCUMIN NPs loaded with Curcumin/IR-780. NPs will be characterized by using UV-Visible spectroscopy, SEM and TEM. Hydrodynamic diameter, polydispersity index (PDI) and zeta potential will be analyzed by Dynamic Light Scattering (DLS). Cellular interactions of the NPs and uptake studies will be carried out on different cancer cell lines using fluorescent microscopy and flow cytometry. The absorption spectra of the NPs will be determined by storing them under dark/day-light conditions to evaluate the photostability of particles using the UV-visible spectrophotometer.

To study the photothermal transduction efficacy, INULIN-PEG-CURCUMIN polymeric-metallic NPs will be irradiated by 808 nm laser for regular time intervals, and the temperature of these nanoparticles will be recorded before and after laser irradiation at standard time intervals. A thermal camera will take thermal images corresponding to various time points. To study the triggered release profile, absorbance and emission of the sample will be taken before and after the laser irradiation. Absorbance will be measured using a UV-VIS spectrophotometer to understand the degradation of IR-780 and the stability of curcumin. The fluorescence of curcumin will be measured at an excitation of 400 nm, and that of IR-780 will be measured at an excitation of 780 nm. The cytotoxicity of curcumin will be evaluated in cancer cell lines, and *in vitro,* biocompatibility studies will be carried out using non-cancerous cell lines by MTT assay.

❖ Limitations of the work:

The following are the limitations of the cancer research work proposed.

One of the fundamental problems in cancer research is finding an effective way to test potential therapies. Without administering the drug to a person with cancer, there's no way to know how a human body will react to a given treatment, nor what impact that treatment will have on the cancer. Chemotherapy drugs and radiation can destroy all types of healthy blood cells and harm the body's production of new ones. Low levels of red blood cells (the cells that carry oxygen) can lead to anaemia, which causes tiredness, paleness, shortness of breath, and a fast heartbeat.

The complications from surgery (e.g. infection, and others that are site-specific), inability to remove cancer in other parts of the body (i.e. metastatic disease), inability to safely remove kill cells in certain parts of the body where radiation therapy may have fewer side effects (e.g. in certain types of brain tumours). Although photothermal therapy has turned out to be a potential pre-clinical chemotherapeutic modality, photothermal therapy still suffers from critical drawbacks, including limited depth of light penetration, low delivery efficiency of photothermal agents, and damage to normal tissues.

❖ Expected Outcomes:

On the successful completion of the proposed work, Inulin can be proved to be an effective carrier molecule for combinational (Bimodal) therapy, including photothermal treatment. Inulin can be used with other drugs for target-oriented anti-cancer therapy and may evolve as a versatile imaging agent. The future focus would involve *in vivo* studies to check the applicability of these nanoparticles.

❖ Proposed Workplan with Tentative Chapters:

❖ **Chapter-1**

Synthesis & Characterization of Curcumin/IR-780 loaded INULIN-PEG polymeric-metallic nanosystem.

❖ **Chapter-2**

Evaluation of cellular uptake, *in vitro* imaging & photothermal transduction efficacy of NPs.

❖ **Chapter-3**

Study of Biocompatibility, PTT-mediated cytotoxicity and drug accumulation/efficacy profile under *in-vivo* conditions.

❖ Year-wise plan of work and Targets achieved:

❖ **<u>First Year</u>:**

Ph.D. course work, Literature survey, NPs synthesis and characterisation.

❖ **<u>Second Year</u>:**

Cancer Theranostics studies such as *in-vitro* and *in-vivo* studies

❖ **<u>Third Year</u>:**

Result writing and data analysis, Publication of research paper, Thesis writing and submission to the university**.**

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